

⑫

**EUROPEAN PATENT APPLICATION**

⑰ Application number: **85305362.7**

⑱ Date of filing: **26.07.85**

⑤① Int. Cl. 4: **G 01 N 33/574, G 01 N 33/76,**  
**G 01 N 33/68, G 01 N 33/573,**  
**G 01 N 33/561**

⑤③ Priority: **07.08.84 JP 165351/84**

⑤④ Date of publication of application: **12.02.86**  
**Bulletin 86/7**

⑤④ Designated Contracting States: **CH DE FR GB IT LI SE**

⑦① Applicant: **Kyowa Medex Co. Ltd., 6-1 Ohtemachi**  
**Itchome Chiyoda-ku, Tokyo (JP)**

⑦② Inventor: **Ishiguro, Tatsuya, 57,**  
**Shimogamomina-kuchi-machi Sakyo-ky, Kyoto-shi**  
**Kyoto-fu (JP)**

⑦④ Representative: **Lambert, Hugh Richmond et al, D.**  
**YOUNG & CO. 10 Staple Inn, London, WC1V 7RD (GB)**

⑤⑤ **Method for diagnosing cancer.**

⑤⑦ Disclosed is a method for diagnosing cancer by qualitative and quantitative determination according to an immuno-serological assay of cancer-related glycoantigens produced in a body fluid by cancerisation or dedifferentiation of normal cells by determination of the bindability of the glycoantigen to lectin.

**EP 0 171 243 A2**

0171243

METHOD FOR DIAGNOSING CANCER

5

10        This invention relates to a method for diagnosing  
cancer by qualitative and quantitative determination according  
to an immuno-serological assay of cancer-related antigens in a  
body fluid, particularly glycoprotein, glycolipid and  
glycoantigen produced by cancerization or dedifferentiation of  
normal cells.

15

Heretofore, specific immuno-serological assay of  
cancer has been carried out according to methods for assaying  
cancer-specific glycoprotein as a cancer-diagnosing marker.  
20        These methods utilize the immunogenicity of protein moiety of  
glycoprotein, where the tumor marker includes, for example,  $\alpha$ -  
fetoprotein (hereinafter referred to as AFP), carcinoembryonic  
antigen (hereinafter referred to as CEA),  $\gamma$ -  
glutamyltranspeptidase (hereinafter referred to as  $\gamma$ -GTP),  
25        human chorionic gonadotropin (hereinafter referred to as HCG),  
etc.

For example, measurement of serum AFP value is known  
as a specific immunoserological diagnosis of primary hepatic  
carcinoma. So long as its increase is small, it is difficult  
30        to decide whether it is caused by the regeneration of  
hepatocyte or by hepatoma cells. AFP considerably increases  
not only in the case of primary hepatic carcinoma, but also  
sometimes in the case of metastasis to the liver from the  
oophoroma, or sometimes from the gastric cancer and the  
35        pancreatic cancer (metastatic hepatic carcinoma), and thus the

0171243

differential diagnosis of cancer is hard to make on the basis of mere quantitative change.

5           Taking into account the facts that cancer-related glycoantigens produced from undifferentiation type cells (particular cancer cells) are released into the body fluid with cell cancerization; the glycoantigens have local differences in the steric structure from those produced from  
10   the normal differentiation type cells; lectin as the glycide-bound protein can specifically recognize the local steric structures of these glycoantigen, the present inventor has found that in a method for assaying the lectin bindability by affinity cross-lined immunoelectrophoresis (hereinafter  
15   referred to as ACIE), the assay accuracy can be improved by high sensitive assay using peroxidase-labelled protein A, 4-methoxy-1-naphthol and hydrogen peroxide in a coloring system after the electrophoresis (the peroxidase is hereinafter referred to as POD), and also the assay time can be shortened.  
20   Furthermore, the present inventor has found that the kind of cancer can definitely be differentiated by properly combining the binding specificities of lectins.

25           The present invention relates to a method for diagnosing cancer by assaying a bindability of lectin to a cancer-related antigen in a body fluid by ACIE, and differentiating the kind of cancer by one of or a combination of at least two of the assay results. Furthermore, the  
30   diagnosis of cancer can be carried out with a higher sensitivity by assay using POD-labelled protein A, 4-methoxy-1-naphthol and hydrogen peroxide in the coloring in the ACIE.

          The body fluid to be used in the present invention is various body fluids including, for example, blood, urine,  
35   saliva, gastric juice, intestinal juice, pancreatic juice,

0171243

bile, cell tissue fluid, edema fluid, ascitic fluid, hydrothorax fluid, hydropericardic fluid, synovial fluid, cerebrospinal fluid, amniotic fluid, genital secretion fluid, mother's milk, nasal mucus, phlegm, tear, sweat, etc., and it is particularly preferable to use blood in the form of serum or plasma.

The cancer-related glycoantigen includes AFP, CEA, HCG,  $\gamma$ -GTP, etc.

The lectin to be used in the present invention includes jack bean lectin (hereinafter referred to as ConA) and lentil lectin (hereinafter referred to as LCH) as lectins capable of being specifically bound to  $\alpha$ -D-glucose and  $\alpha$ -D-mannose; isolectin PHA-E of kidney bean lectin (hereinafter referred to as PHA) as lectin capable of being specifically bound to N-acetylgalactosamine; isolectin RCA-I of castor bean lectin (hereinafter referred to as RCA) as lectin capable of being specifically bound to  $\beta$ -D-galactose; isolectin BSA-II of banderilla bean lectin (hereinafter referred to as BSA) as lectin capable of being specifically bound to N-acetylglucosamine, etc.

In the present method, it is preferable to utilize ACIE method for separation and identification of cancer-related glycoantigens. That is, a sample containing a cancer-related glycoantigen is subjected to one-dimensional electrophoresis separation in a lectin-containing gel, and then the individual separated fractions are subjected to two-dimensional electrophoresis and coloring in an antiserum-containing gel, whereby the individual fractions can be quantitatively determined as sharp precipitate peaks. The lectin bindability can be determined from the specific change in the migration distance.

In the present invention, an enzyme immunoassay is used for the coloring after the ACIE. Particularly, a coloring system comprising POD-labelled protein A, 4-methoxy-1-naphthol and hydrogen peroxide is a clear, rapid and simple

means with a high sensitivity and a high separability, which is very useful for definite diagnosis of cancer.

The diagnosis of cancer may be possible to some extent by a single bindability to lectin, but not  
5 satisfactorily, and it can be made more definitely by a combination of at least two bindabilities to lectin. The combination depends on the nature of assay substance, and, for example, ConA, LCH, etc. are used for the assay of AFP.

More particularly, AFP, a kind of embryonic protein,  
10 is a glycoprotein existing in the blood of patients suffering from primary hepatic carcinoma, gonadal teratoma, or oophoroma. By ACIE, AFP is separated into several fractions due to the difference in bindability to lectin. For example, with ConA, AFP is separated into two fractions, i.e. a ConA-bound fraction and a ConA-unbound fraction. It is  
15 ontogeneologically known that the ConA-bound AFP originates from the hepatocytes and the ConA-unbound AFP originates mostly from the vitellicle. When the serum AFP of patients suffering from the primary hepatic carinoma is assayed in the  
20 present invention, a fraction originating partly from the vitellicle (hereinafter referred to as peak b) is found besides the AFP fraction originating mostly from the embryonic hepatocytes (hereinafter referred to as peak a). In every serum AFP of patients suffering from the metastatic  
25 hepatic carcinoma, the fraction originating from the vitellicle (peak b) is found. With LCH, the embryonic AFP is likewise separated into 4 fractions of peaks A, B, C and D. It is assayed from changes in AFP-producing sites with the elapse of time that peaks A and C are fractions originating  
30 from the hepatocytes and peaks B and D are fractions originating from the vitellicle. It is observed in the AFP fraction patterns of the primary hepatic carcinoma and metastatic hepatic carcinoma that only peaks A and C are found  
in every primary hepatic carcinoma, whereas in the metastatic  
35 hepatic carcinoma, peak B is found besides the peaks A and C.

0171243

That is the AFP fractions produced in the embryonic hepatocytes mainly appear in the case of the primary hepatic carcinoma, whereas in the case of metastatic hepatic carcinoma, a fraction produced in the vitellicle is found  
5 besides the AFP originating from fetal liver. Thus, it is possible to make differential diagnosis between the primary hepatic carcinoma and the metastatic hepatic carcinoma with the guidance of the AFP fraction originating from the vitellicle.

10 Furthermore, with other lectins, i.e. PHA-E, RCA-I and BSA-II, the AFP in the amniotic fluid fails to show a specific behavior with the elapse of time, whereas AFP in the serum of cancer patients shows a specific fraction pattern.

The cancer-related glycoantigen shows a complicated  
15 steric structure, and the individual lectins partially recognize the structure. Thus, no definite diagnosis of cancer can be made from the glycospecific bindability to a single kind of lectin. By an appropriate combination of 5 kinds of lectins, i.e. ConA, LCH, PHA-E, RCA-I, and BSA-II,  
20 the steric structure of a cancer-related glycoantigen can be discriminated more definitely, and more definite differential diagnosis of cancer can be made. The amount of assay sample, the amount of lectin, the amount and proportion of POD-labelled protein A, the amount of substrate, the reaction  
25 time, the temperature and the electrophoresis condition depend on the kind of a substance to be assayed, the kind of lectin, the titer of a receptor to be used, the kind of a carrier, etc., and thus the most appropriate conditions must be experimentally determined for each assay before carrying out  
30 the present method.

Modes of carrying out the present invention will be described in detail below.

A definite amount, for example, at least 5  $\mu$ l, preferably, 5 to 10  $\mu$ l of a body fluid is diffused in a  
35 lectin-containing gel by one-dimensional electrophoresis.

After the one-dimensional migration, each of the separated fractions is subjected to another electrophoresis in the two-dimensional direction in an antiserum-containing gel and to detection by enzyme immunoassay.

5           The lectin concentration depends on the kinds of a substance to be assayed and lectin, and generally the separability depends also on the concentration. The present invention provides a high sensitive assay method which is operable even at a low concentration of lectin such as 200 to  
10       600 µg/ml.

          Any gel carrier is usable without qualification. In the case of two-dimensional ACIE, it is preferable to use agarose having a particularly low electroosmosis owing to less gel deformation. The gel carrier can be prepared according to  
15       a conventional procedure. For example, a predetermined amount of agarose is added to a dilute solution such as distilled water, a barbital buffer solution at a pH of about 8.6 or a Tris-hydrochloric acid buffer solution, and heated at a temperature from 60 to 80°C with gentle stirring, and then the  
20       solution is made to flow onto a desired flat plate, left for cooling and coagulated into a gel state.

          The gel concentration is usually 0.5 to 2.0% by weight, preferably 0.8 to 1.0% by weight, and, if necessary, an antiseptic may be added thereto.

25           When the gel is subjected to electrophoresis for a long time, the gel surface is dried owing to the heat generation, causing a deformation. Furthermore, the binding of lectin to the glycoantigen is generally stronger at a lower temperature, and thus it is preferable to carry out the  
30       electrophoresis at a temperature of not higher than 15°C, preferably 5 to 10°C. For example, in an electronic cooling migration apparatus, efficient separation can be carried out even at a low concentration of lectin.

          The longer is the one-dimensional migration distance  
35       of ACIE, the better is the separation. However, a

0171243

correspondingly larger amount of lectin will be required, and a longer migration time will be required. Thus, at least 6 cm is a sufficient migration distance. As a control marker for the electrophoresis, a coloring matter, e.g. Bromophenol Blue  
5 (hereinafter referred to as BPB)-bound albumin is made to flow in parallel with the assay sample. In the case of AFP, it is sufficient that the BPB-bound albumin can migrate up to about 6 to 7 cm.

On the other hand, the two-dimensional  
10 electrophoresis usually takes a long time such as at least 16 hours, whereas the present assay method has such a high assay sensitivity that migration for 2 to 6 hours, preferably 2 to 4 hours, is enough.

As described above, a cancer-related glycoantigen in  
15 a body fluid can be assayed advantageously by the present invention, thereby differential diagnosis of cancer is possible, and thus the present invention is clinically very useful.

The present invention will be described in detail  
20 below, referring to Examples, but the scope of the present invention will not be restricted by the following examples.

Example 1

Differential diagnosis of hepatic carcinoma by LCH (made  
25 by Pharmacia Fine Chemicals Inc., Sweden)-ACIE:

(a) Preparation of assay samples:

Individual serum of 48 cases of primary hepatic carcinoma and 8 cases of metastatic hepatic carcinoma were diluted with normal human serum (AFP: less than 5 ng/ml) to  
30 make samples at an adjusted AFP concentration of less than 30,000 ng/ml.



0171243

(b) Preparation of LCH-containing agarose plate (for one-dimensional electrophoresis):

To 1.0% agarose (made by FMC Marine Colloids Division, U.S.A., and dissolved in a barbitol buffer solution having pH 8.6 and an ionic strength of 0.05) was added 200 µg/ml LCH and mixed. Then, 15 ml of the LCH-containing agarose gel was poured into a gel bond (made by FMC Marine Colloids Division, 110 x 125 mm), and coagulated to make an LCH-containing agarose plate having a thickness of about 1 mm.

(c) Preparation of anti-AFP serum-containing agarose plate (for two-dimensional electrophoresis):

To 10 ml of 1.0% agarose was added 20 µl of anti-AFP serum (made by DAKO Co., Denmark). Then 10 ml of the anti-AFP serum-containing agarose gel was poured into the same gel bond as above, and coagulated to make an anti-AFP serum-containing agarose plate having a thickness of about 1 mm.

(d) Preparation of diluted POD-labelled protein A solution:

To 20 µl of POD-labelled protein A (made by E.Y. Laboratories, U.S.A.) was added 10 ml of a dilute solution (prepared by dissolving 14.0g of NaCl in 1l of barbitol buffer solution having pH 8.6) to make a 0.2% POD-labelled protein A dilute solution.

(e) Preparation of coloring reagent solution:

Just before application, 200 µl of 4-methoxy-1-naphthol (made by Aldrich Co., U.S.A.), 10 ml of 0.05M Tris buffer solution (a solution made by mixing 12.114 g/l trishydroxymethylaminomethane and 0.1M HCl in a ratio of 100:77, pH 7.6) and a drop of 30% H<sub>2</sub>O<sub>2</sub> were mixed together to make a coloring reagent solution.

(f) Measurement of AFP:

At first, 10 µl of the sample prepared in section (a) was poured into holes (4 mm in diameter) on the LCH-containing agarose plate prepared in section (b), and subjected to one-dimensional electrophoresis. Migration was

0171243

carried out with barbital buffer solution (pH 8.6) at 3 mA/cm for 90 minutes with cooling at 10°C.

As a control, human serum albumin [fraction V, made by Sigma Co., U.S.A., containing 5  $\mu$ l of 0.01% BPB] was used and the about 6 cm-migrated position was marked.

After the one-dimensional electrophoresis, the agarose plate was cut off with width of about 1 cm in parallel to the migrating direction, tightly placed on the anti-AFP-containing agarose plate prepared in section (c), and subjected to two-dimensional electrophoresis in the direction perpendicular to the direction of one-dimension development at 2.5 mA/cm for 3 hours with cooling at 10°C.

After the migration, the plate was treated with an adsorbent pad (made by Nihon Shoji Co., Japan) for 2 to 3 minutes for elimination of unbound substance, and then washed with a phosphate buffer solution (pH 7.2) for 30 minutes. Again, the plate was dried with an adsorbent pad, and 10 ml of POD-labelled protein A prepared in section (c), was poured onto the flat plate. After the reaction for 30 minutes, drying with an adsorbent pad, washing with a phosphate buffer solution (pH 7.2) for 30 minutes and drying with an adsorbent pad were repeated in the same manner as above, and then 10 ml of the coloring reagent solution prepared in section (e) was added thereto. The plate was subjected to reaction for 2 to 3 minutes. After the washing with water for 2 to 3 minutes and drying with an adsorbent pad, a distance from the albumin position obtained by the one-dimensional electrophoresis to a migration peak shown in the two-dimensional electrophoresis was measured.

As a result, typical migration patterns of serums of patients suffering from the hepatic carcinoma by LCH-ACIE are divided into (1) type in which only peak A appears alone (Type I), (2) type in which only peak C appears alone (Type II), (3) type in which two peaks, i.e. peak A and peak C appear (Type III) and (4) type in which peak B appears besides peak A or

0171243

peak C (Type IV).

Type III is further divided into (a) type in which peak A is higher than peak C (Type IIIa), and (b) type in which peak C is higher than peak A (Type IIIb) according to quantitative difference.

Type IV is further divided into (a) type in which two peaks, i.e. peak A and peak B appear (Type IVa), (b) type in which three peaks, i.e. peak A, peak B and peak C appear (Type IVb), and (c) type in which only peak B appears (Type IVc).

As shown in Table 1, AFP in the serums of primary hepatic carcinoma is fractionated into Type I, Type II and Type III, whereas that of metastatic hepatic carcinoma is all fractionated into Type IV. Thus, this is useful for differentiation between the primary hepatic carcinoma and the metastatic hepatic carcinoma.

Table 1

AFP fraction appearance frequency by LCH-ACIE

Fraction pattern	Fraction as appeared	Primary hepatic carcinoma	Metastatic hepatic carcinoma
Type I	A	2 (4.2%)	0 0
Type II	C	6 (12.5%)	0 0
Type III			
Type IIIa	$A \geq C$	21 (43.7%)	0 0
Type IIIb	$A < C$	19 (39.6%)	0 0
Type IV			
Type IVa	A B	0	4 (50.0%)
Type IVb	A B C	0	2 (25.0%)
Type IVc	B	0	2 (25.0%)
		48	8

0171243

## Example 2

Differential diagnosis of hepatic carcinoma by ConA-ACIE:

Assay was carried out for the same serum samples in the same manner as in Example 1, except that a ConA (made by Pharmacia Fine Chemicals)-containing agarose plate (ConA: 600 µg/ml) was used in place of the LCH-containing agarose plate.

As a result, typical migration patterns of serums of patients suffering from the hepatic carcinoma by ConA-ACIE are divided into (1) type in which only peak a appears (Type 1), (2) type in which two fractions of peak a and peak b appear (Type 2), and (3) type in which only peak b appears (Type 3).

As shown in Tables 2 and 3, AFP in the serum of primary hepatic carcinoma is fractionated into Type 1 (only peak a) and Type 2 (peak a > peak b), whereas that in the metastatic hepatic carcinoma is fractionated into Type 2 (peak a < peak b) and Type 3. This is useful for differentiation of the primary hepatic carcinoma and the metastatic hepatic carcinoma.

Table 2

AFP fraction appearance frequency by ConA-ACIE

Fraction pattern	Fraction as appeared	Primary hepatic carcinoma	Metastatic hepatic carcinoma
Type 1	a	30 (62.5%)	0
Type 2	a b	18* (37.5%)	6 (75%)
Type 3	b	0	2 (25%)
		48	8

\* At least two measurements were made for each case, and every case where Type 2 appeared in at least one measurement was classified into Type 2.

0171243

Table 3

Height of peak b and kind of hepatic carcinoma in Type 2  
by ConA-ACIE

		Primary hepatic carcinoma	Metastatic hepatic carcinoma
5	Number of investigation	18	6
	Height of peak b		
10	Average %	$14.1 \pm 3.6^{*1}$	$44.5 \pm 20.2^{*2}$
	Range	8 - 12	17 - 68

\*1 The higher value was adopted where several measurements  
were made for each case.

15 \*2 The lower value was adopted where several measurements  
were made for each case.

Example 3

20 Differential diagnosis of AFP-producing diseases by PHA-  
E-ACIE:

Assay was carried out for the individual serums from  
5 cases of primary hepatic carcinoma, 4 cases of metastatic  
hepatic carcinoma, 4 case of oophoroma, and one case of  
orchioncus in the same manner as in Example 1 except that a  
25 PHA-E (made by E.Y. Laboratories, USA)-containing agarose  
plate (PHA-E: 200  $\mu$ g/ml) was used in place of the LCH-  
containing agarose plate. Migration peaks were named 4  
fractions of peak A, peak B, peak C and peak D on the ground  
of migration distances shown in Table 4.

30

0171243

Table 4  
Classification of individual peaks by migration distances

Peak A	Peak B	Peak C	Peak D
21.0 <	16.0 - 21.0	12.0 - 16.0	12.0 >

(unit mm)

As a result, the migration patterns of serums of patients suffering from various cancers by PHA-E-ACIE are divided into (1) type in which only peak C, only peak D, or two fractions of peak C and peak D appear (Type 1), and (2) type in which two fractions of peak A and peak C, only peak B, two fractions of peak B and peak C; or three fractions of peak B, peak C and peak D appear (Type 2). Type 2 is characterized by appearance of either peak A or peak B (shown as underlined in Table 5).

No specific change between the AFP in amniotic fluid and the pregnancy period was observed in PHA-E-ACIE. However, general tendency was observed with the serums of cancer patients, as shown in Table 5.

Table 5  
AFP fraction appearance frequency by PHA-E-ACIE

Fraction pattern	Appearance frequency	Primary hepatic carcinoma	Metastatic hepatic carcinoma	Oophoroma	Orchioncus
Type 1	C, D, CD	5	0	1	0
Type 2	<u>AC</u> , <u>B</u> , <u>BC</u> , <u>BCD</u>	0	4	3	1
		5	4	4	1

Example 4

Differential diagnosis of AFP-producing diseases by RCA-I-ACIE:

5        Assay was carried out for the same serum samples as  
in Example 3 in the same manner as in Example 1, except that  
an RCA-I (made by E.Y. Laboratories)-containing agarose plate  
(RCA-I: 200 µg/ml) was used in place of the LCH-containing  
agarose plate.

10       Migration peaks were named 4 fractions of peak A,  
peak B, peak C and peak D on the ground of migration distances  
shown in Table 4.

15       As a result, the migration patterns of serums from  
patients suffering from various cancers by RCA-I-ACIE were  
divided into (1) type in which only peak C, only peak D, or  
two fractions of peak C and peak D appear (Type 1); (2) two  
fractions of peak A and peak C, two fractions of peak A and  
peak D, or three fractions of peak A, peak C and peak D appear  
(Type 2); and (3) type in which three fractions of peak A,  
peak B and peak D, or two fractions of peak B and peak D  
20       appear (Type 3). Type 2 and type 3 are characterized by  
appearance of peak A and/or peak B (shown as underlined in  
Table 6).

25       No specific change in the AFP in amniotic fluid and  
the pregnancy period was observed in RCA-I-ACIE. However,  
general tendency was observed in the serums of cancer patients  
as shown in Table 6.

Table 6  
AFD fraction appearance frequency by RCA-I-ACIE

5	Fraction pattern	Appearance frequency	Primary	Metastatic	Oophoroma	Orchioncus
			hepatic carcinoma	hepatic carcinoma		
	Type 1	C, D, CD	5	1	0	0
	Type 2	<u>AC</u> , <u>AD</u> , <u>ACD</u>	0	2	3	1
10	Type 3	<u>ABD</u> , <u>BD</u>	0	1	1	0
			5	4	4	1

15 Example 5

Differential diagnosis of AFP-producing diseases by BSA-II-ACIE:

20 Assay was carried out for the same serum samples as in Example 3 in the same manner as in Example 1 except that a BSA-II (made by E.Y. Laboratories)-containing agarose plate (BSA-II: 200 µg/ml) was used in place of the LCH-containing agarose plate.

25 Migration peaks were named 4 fractions of peak A, peak B, peak C and peak D on the ground of migration distances shown in Table 4.

30 As a result, the migration patterns of serums from patients suffering from various cancers by BSA-II-ACIE were divided into (1) type in which only peak C or only peak D appears (Type 1), and (2) type in which three fractions of peak A, peak B and peak D, or two fractions of peak B and peak D appear (Type 2). Type 2 is characterized by appearance of peak A and/or peak B (shown as underlined in Table 7).

35 No specific change between the AFP in amniotic fluid and the pregnancy period was observed in BSA-II-ACIE. However, general tendency was observed in the serums of cancer



0171243

patients, as shown in Table 7.

Table 7

AFP fraction appearance frequency by BSA-II-ACIE

Fraction pattern	Appearance frequency	Primary	Metastatic	Oophoroma	Orchioncus
		hepatic carcinoma	hepatic carcinoma		
Type 1	C, D	5	1	4	1
Type 2	<u>ABD</u> , <u>BD</u>	0	3	0	0
		5	4	4	1

Example 6

Differential diagnosis of cancer by combination of ConA and LCH:

Cases of obscure differentiation in Example 2, that is, 4 cases showing a high peak b value (14% or higher) in the primary hepatic carcinoma and 6 cases showing a low peak b value (45% or lower) in the metastatic hepatic cancer in the patient serums classified into type 2 by ConA were checked by the results of fraction patterns by LCH (Example 1).

As a result, it was found that all the primary hepatic carcinoma belonged to type III, and that all the metastatic hepatic carcinoma belonged to Type IV.

Example 7

Differential diagnosis of cancer by combination of ConA and PHA-E:

Among the cases of obscure differentiation in Example 2, that is, cases showing a high peak b value (14% or higher) in the primary hepatic cancer and cases showing a low peak b value (45% or lower) in the metastatic hepatic

0171243

carcinoma in the patient serums belonging to type 2 by ConA, a few cases of the patient serums were investigated by PHA-E and the fraction patterns were estimated compared with the results of Example 3.

- 5           As a result, it was found that all the primary hepatic carcinoma belonged to Type 1, and that all the metastatic hepatic carcinoma belonged to Type 2.

Example 8

- 10           Differential diagnosis of cancer by combination of PHA-E and RCA-I:

- Cases of obscure differentiation in Examples 3 and 4, that is, the serums of patients suffering from the oophoroma showing Type 1 by PHA-E were classified into Type 3  
15           by RCA-I.

          The serums of patients suffering from the metastatic hepatic carcinoma showing Type 1 by RCA-I were classified into Type 2 by RHA-E.

20           Example 9

          Differential diagnosis of cancer by combination of PHA-E and BSA-II:

- Cases of obscure differentiation in Examples 3 and 5, that is, serums of patients suffering from the oophoroma  
25           showing Type 1 by PHA-E were classified into Type 1 by BSA-II.

          The serums of patients suffering from the metastatic hepatic carcinoma showing Type 1 by BSA-II were classified into Type 2 by PHA-E.

30           Example 10

          Differential diagnosis of cancer by combination of RCA-I and LCH:

- Cases of obscure differentiation in Example 4, that is, serums of patient suffering from the metastatic hepatic  
35           carcinoma showing Type 1 by RCA-I were classified into Type

IVb by LCH (Example 2).

Example 11

5 Differential diagnosis of cancer by combination of RCA-I  
and BSA-II:

Cases of obscure differentiation in Examples 4 and  
5, that is, serums of patients suffering from the metastatic  
hepatic cancer showing Type 1 by RCA-I were classified into  
Type 2 by BSA-II.

10 Furthermore, the serums of patients suffering from  
the metastatic hepatic carcinoma showing Type 1 by BSA-II were  
classified into Type 3 by RCA-I.

Example 12

15 Differential diagnosis of cancer by combination of BSA-II  
and LCH:

Cases of obscure differentiation in Example 5, that  
is, serums of patients suffering from the metastatic hepatic  
carcinoma showing Type 1 by BSA-II were classified into Type  
20 IVb by LCH (Example 2).

As described above, it is possible to make more  
definite differential diagnosis of cancer by combining 5 kinds  
of lectins, i.e. ConA, LCH, PHA-E, RCA-I and BSA-II  
complementarily as desired.

25

CLAIMS

1. A method of diagnosing cancer in a patient by the immuno-  
serological assay of cancer-related antigens in a body fluid, characterised by  
5 assaying the bindability of a cancer-related glycoantigen in said body fluid  
to lectin by affinity cross-lined immunoelectrophoresis, and combining the  
results of at least two such assays thereby to differentiate the kind of  
cancer.
- 10 2. A method according to claim 1, characterised in that the cancer-  
related glycoantigen assayed is a  $\alpha$ -fetoprotein, human chorionic gonado-  
tropin, carcinoembryonic antigen, or  $\gamma$ -glutamyltranspeptidase.
- 15 3. A method according to claim 1 or 2, characterised in that the lectin  
used in the determination of the bindability thereto of said glycoprotein is  
jack bean lectin, lentil lectin, castor bean lectin, kidney bean lectin, or  
banderilla bean lectin.
- 20 4. A method according to claim 1, 2 or 3, characterised in that after the  
affinity cross-lined immunoelectrophoresis the assay is carried out using  
peroxidase-labelled protein A, 4-methoxy-1-naphthol and hydrogen peroxide.
- 25 5. A method of diagnosing cancer which comprises assaying a bindability  
of a cancer-related glycoantigen in a body fluid to lectin by affinity cross-  
lined immunoelectrophoresis, characterised in that after the affinity cross-  
lined immunoelectrophoresis the assay is performed using peroxidase-  
labelled protein A, 4-methoxy-1-naphthol and hydrogen peroxide.
- 30 6. A method according to claim 5, characterised in that the cancer-  
related glycoantigen assayed is  $\alpha$ -fetoprotein, human chorionic gonado-  
tropin, carcinoembryonic antigen, or  $\gamma$ -glutamyltransferase.
- 35 7. A method according to claim 5 or 6, characterised in that the lectin  
used in the determination of the bindability thereto of said glycoprotein is  
jack bean lectin, lentil lectin, castor bean lectin, kidney bean lectin, or  
banderilla bean lectin.



Europäisches Patentamt  
European Patent Office  
Office européen des brevets

(11) Publication number:

**0 171 243  
A3**

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 85305362.7

(22) Date of filing: 26.07.85

(51) Int. Cl.: **G 01 N 33/574**, G 01 N 33/76,  
G 01 N 33/68, G 01 N 33/573,  
G 01 N 33/561

(30) Priority: 07.08.84 JP 165351/84

(43) Date of publication of application: 12.02.86  
Bulletin 86/7

(84) Designated Contracting States: CH DE FR GB IT LI SE

(88) Date of deferred publication of search  
report: 27.07.88 Bulletin 88/30

(71) Applicant: Kyowa Medex Co. Ltd., 6-1 Ohtemachi  
Itchome Chiyoda-ku, Tokyo (JP)

(72) Inventor: Ishiguro, Tatsuya, 57,  
Shimogamominakuchi-machi Sakyo-ky, Kyoto-shi  
Kyoto-fu (JP)

(74) Representative: Lambert, Hugh Richmond et al, D.  
YOUNG & CO. 10 Staple Inn, London, WC1V 7RD (GB)

(54) Method for diagnosing cancer.

(57) Disclosed is a method for diagnosing cancer by qualitative and quantitative determination according to an immuno-serological assay of cancer-related glycoantigens produced in a body fluid by cancerisation or dedifferentiation of normal cells by determination of the bindability of the glycoantigen to lectin.

**EP 0 171 243 A3**



Europäisches Patentamt  
European Patent Office  
Office européen des brevets

(11) Publication number:

**0 171 243  
A3**

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 85305362.7

(22) Date of filing: 26.07.85

(51) Int. Cl. 4: **G 01 N 33/574**, G 01 N 33/76,  
G 01 N 33/68, G 01 N 33/573,  
G 01 N 33/561

(30) Priority: 07.08.84 JP 165351/84

(43) Date of publication of application: 12.02.86  
Bulletin 86/7

(64) Designated Contracting States: CH DE FR GB IT LI SE

(88) Date of deferred publication of search  
report: 27.07.88 Bulletin 88/30

(71) Applicant: **Kyowa Medex Co. Ltd.**, 6-1 Ohtemachi  
Itchome Chiyoda-ku, Tokyo (JP)

(72) Inventor: **Ishiguro, Tatsuya**, 57,  
Shimogamominakuchi-machi Sakyo-ky, Kyoto-shi  
Kyoto-fu (JP)

(74) Representative: **Lambert, Hugh Richmond et al**, D.  
**YOUNG & CO.** 10 Staple Inn, London, WC1V 7RD (GB)

(54) **Method for diagnosing cancer.**

(57) Disclosed is a method for diagnosing cancer by qualitative and quantitative determination according to an immuno-serological assay of cancer-related glycoantigens produced in a body fluid by cancerisation or dedifferentiation of normal cells by determination of the bindability of the glycoantigen to lectin.

**EP 0 171 243 A3**



European Patent  
Office

# EUROPEAN SEARCH REPORT

0171243

Application Number

EP 85 30 5362

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	CHEMICAL ABSTRACTS, vol. 96, no. 11, 15th March 1982, page 283, abstract no. 82101c, Columbus, Ohio, US; J. MIYAZAKI et al.: "Lectin affinities of alpha-fetoprotein in liver cirrhosis, hepatocellular carcinoma and metastatic liver tumor", & KANZO 1981, 22(11), 1559-68 * Whole abstract *	1-3	G 01 N 33/574 G 01 N 33/76 G 01 N 33/68 G 01 N 33/573 G 01 N 33/561
Y	IDEM ---	4-7	
X,P	CANCER, vol. 55, no. 1, 1st January 1985, pages 156-159; T. ISHIGURO et al.: "Serum alpha-fetoprotein subfractions in patients with primary hepatoma or hepatic metastasis of gastric cancer" * Whole document *	1-3	
X,O	ELECTROPHOR. '83, ADV. METHODS, BIOCHEM. CLIN. APPL., PROC. INT. CONF., 1983 (published 1984), pages 611-618, Walter de Gruyter & Co.; K. TAKETA et al.: "Separation and identification of different molecular species of human alpha-fetoprotein by double affinity electrophoresis with concanavalin A and lens culinaris hemagglutinin A" * Whole document *	1-3	TECHNICAL FIELDS SEARCHED (Int. Cl.4)  G 01 N
Y	IDEM --- -/- -	4-7	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 22-04-1988	Examiner HITCHEN C.E.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	



European Patent  
Office

# EUROPEAN SEARCH REPORT

Page 2  
0171243  
Application Number

EP 85 30 5362

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	CHEMICAL ABSTRACTS, vol. 102, no. 9, 4th March 1985, page 265, abstract no. 75036z, Columbus, Ohio, US; T. ISHIGURO et al.: "Alpha-fetoprotein subfractions in serums from patients with low alpha-fetoprotein-producing hepatoma identified by the highly sensitive lectin affinity crossed-line immunoelectrophoresis with internal standard system", & IGAKUNO AYUMI 1984, 131(6), 384-8 * Abstract *	4-7	
A	IDEM ---	1-3	
X,0	CHEMICAL ABSTRACTS, vol. 101, no. 1, 2nd July 1984, page 420, abstract no. 4856k, Columbus, Ohio, US; K. TAKETA et al.: "Molecular species of human alpha-fetoprotein and gamma-glutamyltransferase more specific to hepatocellular carcinoma; demonstration by differential affinities to lectins", & PROTIDES BIOL. FLUIDS 1983 (Pub. 1984), 31, 387-90 * Abstract * --- -/-	1-3	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 22-04-1988	Examiner HITCHEN C.E.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			





European Patent  
Office

# EUROPEAN SEARCH REPORT

0171243

Application Number

EP 85 30 5362

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X,D	CHEMICAL ABSTRACTS, vol. 95, no. 15, 12th October 1981, page 291, abstract no. 128616j, Columbus, Ohio, US; J. BREBOROWICZ et al.: "Application of lectin affinity electrophoresis for studies of microheterogeneity of human alpha-fetoprotein", & LECTINS: BIOL., BIOCHEM., CLIN. BIOCHEM., PROC. LECTIN MEET., 3rd 1980 (Pub. 1981), 1, 303-14 * Abstract *	1-3	
A	US-A-4 455 380 (M. ADACHI) * Abstract; columns 1-2 * -----	2	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 22-04-1988	Examiner HITCHEN C.E.
<b>CATEGORY OF CITED DOCUMENTS</b>			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	

EPO FORM 1503 03/82 (P0401)